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The research objective of this project is to investigate the importance of microbial processes in hydrogen embrittlement of metals. During the past year we have used a sophisticated adaptation of the Devanathan cell to quantify bacterial hydrogen permeation through a defined metal. We found that hydrogen production under bacterial films is sufficient to produce permeation currents equivalent to measured thresholds for hydrogen-induced cracking of steels. During the next year we will stress metals in the presence of biofilms of hydrogen-producing bacteria in order to measure directly damage to metals caused by bacterial hydrogen.

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ANNUAL REPORT: CONTRACT N0014-88-K-0121

PRINCIPAL INVESTIGATOR: Ralph Mitchell

CO-INVESTIGATOR: Tim Ford

CONTRACTOR: Harvard University

CONTRACT TITLE: The Role of Microorganisms in Marine Corrosion

RESEARCH OBJECTIVE: To investigate the role of microorganisms in hydrogen embrittlement. Specific objectives include, 1. Quantification of amounts of microbially produced hydrogen absorbed by sensitive metals, 2. Effect of competition for microbially produced hydrogen between a metal surface and hydrogen consuming bacteria, 3. Effect of microbial metabolites on hydrogen absorption by metals.

PROGRESS (Year 2): We have adapted a conventional electrochemical technique to quantitatively assay hydrogen production under microbial films. Our research has focused on defined metal foils, specifically palladium. The advantage of using palladium is that hydrogen permeation is not complicated by secondary phases within the metal and absorption efficiency is very high. Hydrogen permeation has been quantified with pure cultures of bacteria using the defined system, allowing calculations of hydrogen production on a per cell basis. Maximum production of 6.5×10^{-11} mol per cell was calculated.

Direct counts of bacteria on the palladium surface do not indicate formation of a thick bacterial film (probably a function of the rich nutrient media). However, those organisms that have attached to the palladium appear to be particularly metabolically active, fermenting glucose at a rate at least 10 fold the rate of suspended cells. Two possible explanations for this high fermentation rate are: 1. The attached cells are metabolically more active than suspended cells due to accumulation of nutrients at surfaces and the interactions within a microbial film. 2. Absorption of atomic hydrogen by the palladium foil represents removal of an otherwise inhibitory metabolic by-product.

Table 1 lists permeation and growth parameters of *C. acetobutylicum*, *Clostridium butyricum* and *Desulfovibrio sulfuricans* ssp. *aesturii*. Our data showed that although maximum hydrogen permeation was lower for *C. butyricum* than *C. acetobutylicum*, total hydrogen was reasonably high despite lower numbers of attached bacteria. This reflects the different metabolism of *C. butyricum*, resulting in hydrogen production over a longer period (Figure 1). Hydrogen production by *D. sulfuricans* was found to be negligible despite considerable numbers of organisms attached to the input surface.

Many factors can influence absorption of microbially-produced hydrogen into a metal. It is only by using a system

Bacteria	Maximum permeation (uA/cm^2)	Total Hydrogen (umol)	Most Cathodic Input Surface Pot. (mv vs SCE)	Metabolites Ac (mM)	Metabolites Bu (mM)	Numbers $/cm^2$ ($x\ 10^6$)
Clostridium acetobutylicum	421.9	144.6	-477	21.3	24.8	2.4
Clostridium butyricum	158	102.9	-477	21.9	25.5	0.3
Desulfovibrio desulfuricans	0.7	-	-482	2.9	0.6	7.4

Table 1. Permeation characteristics, attached numbers and volatile fatty acid production of Clostridium acetobutylicum, Clostridium butyricum and Desulfovibrio desulfuricans.

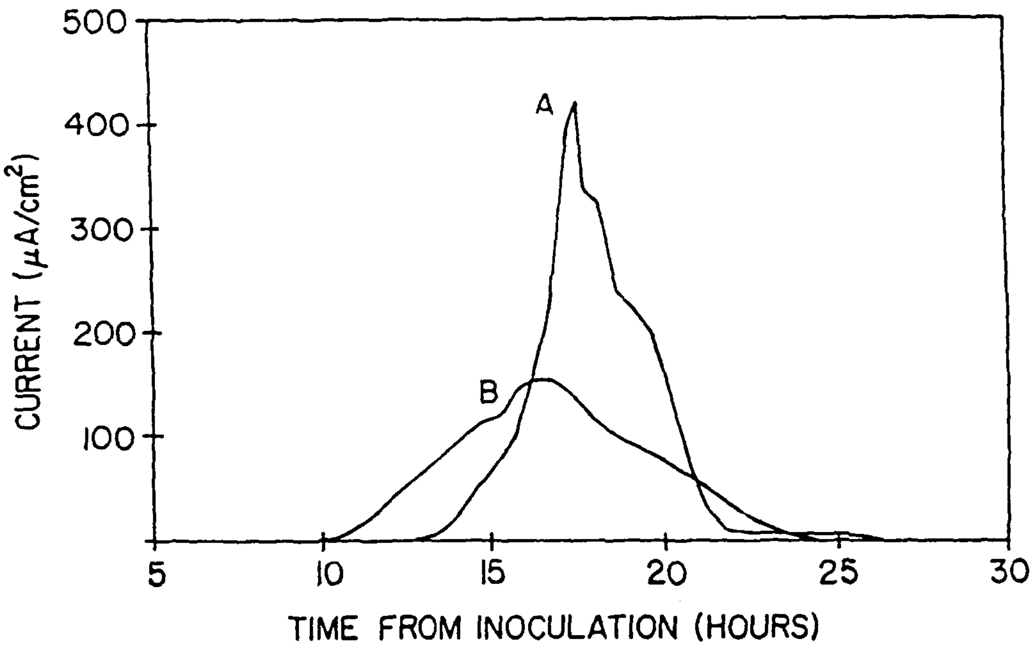

Figure 1. A: Hydrogen permeation transient for C. acetobutylicum.

B: Hydrogen permeation transient for C. butyricum.

such as palladium, where absorption efficiency is high and permeation behavior is well-characterized, that hydrogen production rates can be readily quantified. The permeation characteristics of C. butyricum reflect metabolic processes which differ from the other bacterial species, C. acetobutylicum. We hypothesize that in the latter case, the rapid decrease in hydrogen permeation current after the maximum was reached reflects a change in metabolism from production of hydrogen to utilization. In the case of C. butyricum, a much slower decline in hydrogen permeation reflects only the stationary growth phase and subsequent sporulation.

The Desulfovibrio data indicate that hydrogen is very tightly cycled in sulfate reduction; no net hydrogen production was observed. Formation of a thick layer of ferrous sulfide on the palladium membrane also inhibited hydrogen permeation when the solution was subsequently saturated with 5% hydrogen gas.

These data were used to assess the possibility of embrittlement of susceptible materials by microbially-produced hydrogen. We calculated the input concentration of hydrogen to palladium from the permeation current density using Fick's first law. We found that the effective hydrogen concentration beneath our bacterial films was close to the concentration required to produce crack propagation in high strength steel. In addition, hydrogen gas pressure under individual bacterial cells may be much higher than the average pressure derived from the total membrane surface.

The large permeation currents measured through the use of palladium permit in-depth analysis of the relationship between the activities of bacteria in biofilms and hydrogen absorption. The modified Devanathan cell provides new insights into the role of bacteria in hydrogen embrittlement processes of alloys in contact with aqueous environments.

We have set up two stress testing systems to study the surface characteristics and failure rates of steels exposed to cultures of hydrogen-producing bacteria. The first system is designed to test stainless steel wire under variable loading conditions. A weight is suspended vertically from one end of the wire which passes through a microbiological cell. Initial studies have shown a considerable decrease in the open circuit potential of the metal on exposure to a clostridial culture. The other system is designed to test steel rods that are stressed close to their maximum tensile strength (as in prestressed concrete). The rod is placed horizontally and a weight is suspended at a predetermined distance from the microbiological cell in order to create the desired stress at a specific point on the upper surface of the rod. Preliminary tests have only been conducted with sterile microbiological media.

OBJECTIVES FOR THE NEXT YEAR:

We will continue to use the adapted Devanathan cell to study the effects of bacterial metabolites on hydrogen permeation through metals. We will also use the cell to study the effect of mixed cultures on hydrogen permeation, particularly in relation to

interspecific hydrogen transfer. The major emphasis of our research will be to use the stress testing systems we have designed to measure actual effects of hydrogen production on metals. To this end, we will stress metals in the presence of hydrogen-producing bacteria and measure damage electrochemically. Metallurgical methods will then be used to analyze crack initiation and propagation.

PUBLICATIONS AND RESEARCH ABSTRACTS:

1. Ford, T.E. and R. Mitchell. 1989. Hydrogen embrittlement: a microbiological perspective. CORROSION/89, Paper No. 189, National Association of Corrosion Engineers, New Orleans, LA.

2. Mitchell, R. and T. Ford. 1989. Surface microbiology and corrosion processes. In: 'Advances in Microbial Corrosion Research' CORROSION/89, National Association of Corrosion Engineers, New Orleans, LA.

3. Ford, T.E. and R. Mitchell. 1989. Electrochemical evaluation of hydrogen embrittlement by microorganisms. 'The Electrochemical Society,' 175th meeting, Los Angeles, CA.
4. Black, J.P., T.E. Ford and R. Mitchell. 1989. Manganese-binding by exopolymers of Deleya marina. 'The American Society for Microbiology' annual meeting, New Orleans, LA.

5. Walch, M., T.E. Ford and R. Mitchell. 1989. Influence of hydrogen-producing, anaerobic bacteria on hydrogen uptake by steel. Corrosion 45:705-709.

6. Henry, E., Ford, T.E. and R. Mitchell. 1989. Hydrogen production in anaerobic biofilms. 'FEMS symposium on microbiology and biochemistry of strict anaerobes involved in interspecific hydrogen transfer.' Marseille, France.

7. Ford, T.E. and Mitchell. The ecology of microbial corrosion. In Marshall, K.C. (ed.), Advances in Microbial Ecology, Vol. 11. (In Press).

8. Ford, T.E., P.C. Searson, T. Harris and R. Mitchell. Investigation of microbiologically-produced hydrogen permeation through palladium. Journal of the Electrochemical Society. (In Press).

INVENTIONS:

None

TRAINING ACTIVITIES:

Two graduate students have been working on the project

WOMEN AND MINORITIES:

One

NON-CITIZENS:

One

AWARDS:

None
No minority or foreign students at present